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Immunotherapy with *Mycobacterium vaccae* as an addition to chemotherapy for the treatment of pulmonary tuberculosis under difficult conditions in Africa

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A study to assess the impact of immunotherapy with *Mycobacterium vaccae* on the treatment of pulmonary tuberculosis was conducted under existing conditions in Kano, a large city in Northern Nigeria. Whilst it did not quite meet all the criteria of a well-controlled randomized or double-blind trial, the study produced results suggestive of a successful intervention. Immunotherapy with *M. vaccae* had a beneficial influence on clinical recovery and survival, whether given after 1, 2 or 3 weeks of chemotherapy, according to an assessment made 10–14 months after treatment.

Approximately 3 weeks (19·8 days) after the onset of chemotherapy (SHRZ), 73% of the patients who received immunotherapy and 19% of those who received placebo (chemotherapy alone) had become sputum negative by microscopy for acid-fast bacilli (AFB). Similarly, a mean fall in erythrocyte sedimentation rate (ESR) of $25{\cdot}4 \pm 2{\cdot}50$ mm and $4{\cdot}0 \pm 2{\cdot}29$ mm was observed in the immunotherapy and placebo recipients respectively, at the same time of assessment. When weight was assessed in the two groups, it was observed that 3 weeks after starting chemotherapy, the recipients of immunotherapy had a mean weight gain of $2{\cdot}90 \pm 0{\cdot}24$ kg whilst placebo recipients had a mean weight gain of only $0{\cdot}55 \pm 0{\cdot}17$ kg.

These parameters were re-evaluated, 10–14 months later. They showed that 11% of the recipients of the active intervention and 84·6% of placebo recipients still had demonstrable AFB in their sputum. The mean weight gain had increased to $7{\cdot}91 \pm 1{\cdot}03$ kg and $2{\cdot}04 \pm 0{\cdot}94$ kg in the immunotherapy and placebo recipients respectively. The recorded mortality amongst those traced in this second follow-up was 40% for the placebo recipients and 0% for the recipients of immunotherapy.

The impact of immunotherapy is discussed against the backdrop of a high mortality rate from tuberculosis, resulting from the absence of the most basic of anti-TB medication in the hospital, a preponderance of fake drugs in the open markets and local chemist stores as well as the rising seroprevalence of HIV and AIDS.

Introduction

The incidence of tuberculosis is high throughout Africa, especially in the sub-Saharan region, where the situation is rapidly worsening (1,2). Current problems affecting the control of tuberculosis include the world recession which is limiting funds available for health care (3), and the epidemic of human immunodeficiency virus (HIV) infection acting as a major risk factor for tuberculosis (4–6). Thus national health services are unable to meet the demands of a rising incidence of tuberculosis with the long treatment period it requires. The situation would be altered radically if the period of chemotherapy could be reduced by the introduction of an immunotherapeutic step, obviating the need for the continuation phase of treatment (7). The continuation phase of therapy is aimed at overcoming persister bacilli, which are in a state of altered metabolism unaffected by drugs. This persistence is partly due to the nature of the organism and partly to the failure of the host's immune mechanisms to return to a state of antibacterial immunity, even

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after substantial reduction of bacillary and antigenic loads.

Preliminary studies in the Middlesex Hospital, London (8), Kuwait (9,10), and The Gambia (11) indicated that injection of a suspension of killed *Myobacterium vaccae* 4–6 weeks after the onset of chemotherapy, may have the effects required of a potential immunotherapeutic agent (7,12). In this study, we have assessed this preparation under the typical adverse conditions prevalent in regions where such therapy is needed most. The normal practices of the investigating institution were disrupted as little as possible, the exceptions being the administration of immunotherapy or placebo, additional investigations, and attempting follow-up about 1 yr after diagnosis.

The city of Kano on the edge of the Sahel in Northern Nigeria has a population of about three million, and tuberculosis is a common disease. The state provides free anti-tuberculosis chemotherapy when available. However, the Infectious Diseases Hospital frequently has no stocks, with the occasional exception of streptomycin. Thus, the prescribed medication is frequently bought from private pharmacies at prices usually beyond the patients' means. The quality of purchased drugs is uncertain and they are often fake, diluted, or outdated (3,13,14). This results in many patients receiving treatment for only a few weeks, and very high rates of treatment failure and death are observed.

Materials and Methods

PATIENTS

The patients used in this study were those who presented at the Infectious Diseases Hospital, Kano, with symptoms of pulmonary tuberculosis, and in whose unconcentrated sputum, acid-fast bacilli could be seen after staining by the Ziehl–Neelsen method. All were aged 18 years or above and entered the study between December 1990 and April 1991 after informed consent. All patients were counselled and gave their consent prior to HIV testing. Each patient was weighed and clinically examined, and chest X-ray was obtained from 75 patients. The limitation was the availability of X-ray films. The majority of patients who had chest X-ray examinations showed evidence of advanced bilateral tuberculosis, mostly with cavities. Gross weight loss and a history of chronic cough and haemoptysis were common at presentation. Many were brought to hospital having collapsed at home or on the streets. Blood samples were taken for blood count and erythrocyte sedimentation rate (ESR), and serum was stored at $-20°C$ for later serological studies. Each patient was prescribed a 2 month course of daily chemotherapy with streptomycin (1 g IM), rifampicin (450 mg), isoniazid (300 mg) and pyrazinamide (1·5 g), followed by a daily 6 month continuation phase of rifampicin (450 mg), and isoniazid (300 mg). Most patients could afford only a few weeks' supply of drugs at a time. Patients who did not receive daily chemotherapy from diagnosis to the point at which immunotherapy or placebo was instituted, were excluded from the study. One hundred and eighty randomized patients were recruited. Due to their level of debility, 60 patients (43 placebo recipients and 17 *M. vaccae* recipients) from the 180 patients who were randomized, remained in hospital for 1–2 weeks, but most recruited patients were given ambulatory care. This did not have implications for their therapy, since at this point the hospital did not have any stocks of anti-TB drugs except for streptomycin. Dependent upon supplies, some patients benefited from a variable amount of free streptomycin and others did not. However, all recruited patients who did not receive the free supply of streptomycin were made to purchase at least 1 month's worth from the local chemist shops. Consequently, inpatient care did not result in privileged chemotherapy. These inpatients, like their counterparts who received ambulatory treatment, purchased drugs from local pharmacy stores and thus had equal chances of obtaining fake medications. It is important to note that prior to recruitment into the study, pill-counting ensured that patients had at least 1 month's worth of SHRZ.

STUDY DESIGN

The study was a single-blind randomized placebo controlled trial. The difference in the inflammatory response provoked by the placebo (normal saline) and the *M. vaccae*, and the logistical requirements of a double-blind trial, argued in favour of a single-blind trial. Clinical assessment was by the principal investigator (PCO) and two other medical officers. All three were generally unaware of the grouping, but sometimes the site of injection will have been seen. However, the endpoints of the trial were assessed by health care workers who were definitely blind to the intervention given. Thus, microbiological examination of sputum, ESR estimation, follow-up patient tracing and weight measurements were carried out by staff unaware of which patients had received the active preparation.

Primary outcome

Sputum smear conversion at 1 month and at 1 yr was considered a realistic outcome, in view of the fact

that the hospital had previously recorded extremely poor rates and did not have any culture facilities.

Secondary outcomes

These included: (a) fall in ESR, (b) decrease in tuberculosis-associated mortality, (c) resolution of chest X-ray, (d) fall in % agalactosyl IgG, (e) weight change from values at diagnosis, (f) resolution of presenting clinical signs and symptoms.

INTERVENTION

The immunotherapy consisted of an autoclaved suspension of 10 mg wet weight *M. vaccae* NCTC 11659 ml^{-1} of M/15 borate-buffered saline pH 8·0 (10). This was stored at +4°C, and the 0·1 ml dose of suspension was injected intradermally over the deltoid muscle. Normal saline was used as placebo and similarly administered.

Randomization was achieved by drawing a card from a black bag. Intradermal injection of immunotherapy was given after 1 ($n=57$), 2 ($n=26$) or 3 ($n=7$) weeks of chemotherapy, and similar numbers of patients received injections of saline. It was realized from the beginning that follow-up would be difficult, and that far more patients would enter the study than could be expected to be followed-up.

FOLLOW-UP

When possible, 1–2 weeks after intervention, patients were examined and weighed, second blood samples were obtained for ESR and serology, and sputa were taken for microscopy (see results). It is also the routine practice of the hospital to repeat the chest X-ray, 1 month after diagnosis if film is available, and this was done for 35 of the randomized patients seen in the first follow-up in each of the two groups.

Other than occasional attendance as out-patients thereafter, patients were not followed-up again until December 1991 to April 1992, some 10–14 months after entering the study. It is therefore not possible to be sure of compliance in the interval between the two evaluations, a common situation in studies involving tuberculosis patients in most parts of Africa. Between entry into the study and the final follow-up, serious ethnic and religious disturbances occurred in Kano, resulting in many people fleeing the city and not returning. Without knowing who had received immunotherapy or placebo, a small local team of medical assistants (led by TA) actively sought the named patients in the city, for most of which postal addresses do not exist. The fate of 34 who had received immunotherapy, and 47 in the placebo group were determined, and those living were brought to hospital for assessment. This consisted of weighing, clinical examination, sputum examination for acid-fast bacilli, and obtaining a final blood sample for ESR and serology from a proportion of the patients. A chest X-ray examination was performed in 20 of the followed-up patients (eight immunotherapy and 12 placebo recipients). Further follow-up is unlikely to be possible since most patients have either gone back to the rural areas, or have relocated elsewhere on account of fear of further religious violence.

BACTERIOLOGY

This was carried out in the routine way by staff who were unaware which patients were in the study, or who had received which intervention. Sputum samples were obtained on 3 consecutive days at the time of first attendance and thereafter, 1–2 weeks after intervention and at the final follow-up. Smears of sputum on microscope slides were heat-fixed and stained for acid-fast bacilli by the Ziehl–Neelsen method. These were examined microscopically under the × 100 objective and recorded as 0–4+ of bacilli.

SEROLOGY

Stored sera were examined by ELISA for antibodies to HIV 1 and 2 (Wellcozyme, Wellcome Laboratories, UK) and for the percentage of agalactosyl IgG [Gal(0)] (15). The assay for Gal (0) estimates the percentage of IgG which lacks terminal galactose from the oligosaccharides situated on the CH_2 domain. It does not measure the total concentration of this glycoform. The %Gal(0) was corrected for age using previously published values (16). The assay method and characteristics have been described elsewhere (15). Stocks of sera remain for future studies.

STATISTICAL ANALYSIS

Fisher's exact test, the Mann–Whitney *U*-test and the paired *t*-test were used for comparison of the difference between means of the immunotherapy and placebo groups.

Results

Since the results were similar, irrespective of timing of the intervention, these are not shown separately.

FIRST FOLLOW-UP ASSESSMENT

Of the 142 patients who returned for assessment 1–2 weeks after injection (Table 1), 77/90 (85·5%) were seen from the immunotherapy group after

Table 1 Results obtained at first follow-up

	Immunotherapy		Placebo
	19·8 ± 8·3 days of chemotherapy		20 ± 7·3 days of chemotherapy
Increase in body weight	2·90 ± 0·24 (n=77)	P<0·001*	0·55 ± 0·17 (n=65)
Paired t-test	P<0·001		n.s.
Mean fall in ESR	25·4 ± 2·50 (n=77)	P<0·001*	4·0 ± 2·9 (n=63)§
Paired t-test	P<0·0001		n.s.
Sputum still +ve for AFB	20/75‡ (26·6%)	P<0·00001†	53/65 (82%)
Change in %Gal(0)	3·61 ± 0·91 (n=28)	P<0·05*	+0·11 ± 1·35 (n=17)
Paired t-test	P<0·0005		n.s.

*Mann–Whitney U-test, †Fisher's exact test, ‡Two patients with missing sputum results excluded, §Two patients with missing ESR results excluded.

means of 19·8 ± 8·3 days since starting chemotherapy, and 9·0 ± 5·6 days since intervention (range 4–34 days). From the placebo group, 65/90 (72%) were seen after 20 ± 7·3 days of chemotherapy, and 12·5 ± 6·8 days since intervention (range 6–30 days). Thus, both the duration and type of chemotherapy received by the two groups were the same at this first assessment (daily SHRZ). At this time, four patients among the placebo recipients had already died whilst in hospital.

10–14 MONTH FOLLOW-UP, AND MORTALITY

At the 10–14 month follow-up, 47 (52%) of the placebo group were located, whether live (31%) or dead. Four of this group had died while in hospital, and 15 more deaths were established, giving a total of 19 deaths amongst the 47 traced, equivalent to 40% mortality. Since there were originally 90 patients in this group, the *recorded* mortality rate, if by chance all dead patients were located, is 19/90, or 21%. Even this recorded mortality rate is in striking contrast to the findings in the immunotherapy group. Of this group, 34 (37%) were traced, and no patient amongst the immunotherapy recipients had died. The survivors traced in each group (28 placebo and 34 immunotherapy recipients) were re-examined.

CLINICAL ASSESSMENT

At diagnosis, all patients had the signs and symptoms of severe tuberculosis, 18 of them with pleural effusions (10 in the immunotherapy group). The majority of the patients had palpable, and sometimes matted cervical lymph nodes, but only 31 had generalized lymphadenopathy (17 in the immunotherapy group). None of the patients had *lupus vulgaris*. Splenomegaly was present in 43 patients and 18 had mild–moderate hepatomegaly (19 and 11 respectively, in the immunotherapy group); these are common findings in a region where malaria and many other infections are endemic.

At the 10–14 month follow-up, the group receiving immunotherapy showed abatement of fever, little or no residual cough, resolution of lymph nodes (even in those found to be seropositive for HIV), no chest pain, and a general improvement in well-being. Among the 34 patients in the immunotherapy group, three had pleural effusion, two had generalized lymphadenopathy, eight had splenomegaly and one had hepatomegaly. On the other hand, most of the placebo recipients had persistence of clinical features of tuberculosis, which often included persistent pleuritic pain, fever and productive cough with sputum occasionally bloodstained. Of the 28 patients followed-up in the placebo group, six had pleural effusions, 10 had generalized lymphadenopathy, 18 had splenomegaly and five had hepatomegaly; significantly more with lymphadenopathy (P<0·004) and splenomegaly (P<0·002) than the immunotherapy group.

On auscultation, the immunotherapy recipients had improved air entry, and only occasional basal crepitations, whereas the placebo group, in general, still had signs of diminished air entry and multiple coarse crepitations all over the lung fields.

Table 2 Results obtained at the second follow-up

	Immunotherapy (n=34)		Placebo (n=47)
Mortality among patients traced	0/34 (0%)	$P<0{\cdot}00001$†	19/47 (40%)
Increase in body weight	7·91 kg (n=33)	$P<0{\cdot}003$*	2·04 kg (n=26)
Mean fall in ESR	42 mm (n=33)	$P<0{\cdot}001$*	14·96 mm (n=26)
Sputum still +ve for AFB	11/33 (33%)	$P<0{\cdot}00002$†	22/26 (84·6%)
HIV1+ve	5/34 (14·7%)		9/47 (19·1%)
Mortality	0/5 (0%)	$P<0{\cdot}03$†	6/9 (67%)
Sputum still +ve for AFB	0/5 (0%)	$P<0{\cdot}018$†	3/3 (100%)

*Mann–Whitney U-test, †Fisher's exact test.

BODY WEIGHT

The mean body weight at the time of diagnosis of those receiving immunotherapy was $47{\cdot}8 \pm 1{\cdot}01$ kg, and of those receiving placebo it was $46{\cdot}8 \pm 1{\cdot}05$ kg (n.s.). After $19{\cdot}8 \pm 8{\cdot}3$ days (immunotherapy group) and $20 \pm 7{\cdot}3$ days (placebo group) of chemotherapy, these weights had changed to $50{\cdot}5 \pm 1{\cdot}09$ kg and $46{\cdot}2 \pm 1{\cdot}19$ kg respectively ($P<0{\cdot}001$ for the difference, see Table 1). The paired t-test showed the change to be significant in the immunotherapy group ($P<0{\cdot}0001$), but not in the placebo group. By the 10–14 month follow-up, survivors had a mean weight of $57{\cdot}03 \pm 1{\cdot}94$ kg in the immunotherapy group, and $51{\cdot}0 \pm 1{\cdot}69$ kg in the placebo group ($P<0{\cdot}0005$ for the difference); paired t-tests showed the weight change to be significant at $P<0{\cdot}0001$ and $P<0{\cdot}04$ with regard to the initial mean weight of immunotherapy recipients and placebo recipients respectively.

CHEMOTHERAPY PURCHASED

Despite prescribing the same chemotherapy, and encouraging its purchase equally, questioning patients 10–14 months after treatment revealed immunotherapy recipients to have bought chemotherapy for an average of 2·9 months, whereas surviving placebo recipients bought it for 6·8 months. We cannot be sure whether this reflects purchase of all drugs prescribed, or just the cheaper ones. Furthermore, these drugs would have been taken intermittently as the patients would not have been able to afford more than a few weeks at a time. All patients purchased drugs from local chemist shops. We do not know the quality of drugs purchased, an important consideration, since more than 50% of the available drugs in circulation are counterfeit (3,13,14). However, each patient had an equal chance of purchasing either fake or genuine drugs.

SPUTUM SMEAR MICROSCOPY

All patients were sputum smear positive for acid-fast bacilli at the time of diagnosis. After $19{\cdot}8 \pm 8{\cdot}3$ days of starting chemotherapy, 73% of the patients who had received immunotherapy were sputum negative. Of those that received the placebo, 19% (12/65) were sputum negative after $20 \pm 7{\cdot}3$ days of chemotherapy. The chemotherapy received was the same for both groups. After 10–14 months, 67% (22/33) of immunotherapy recipients and 15% (4/26) of placebo recipients were smear negative (Table 3). All patients who were sputum positive at the last follow-up, were also positive at the first follow-up examination.

ERYTHROCYTE SEDIMENTATION RATE

At the first follow-up examination after intervention, patients who had received immunotherapy showed a mean fall in ESR of $25{\cdot}4 \pm 2{\cdot}50$ mm compared to that at diagnosis (paired t-test $P>0{\cdot}0001$). In the placebo recipients, a drop in ESR of $4{\cdot}0 \pm 2{\cdot}29$ mm was observed ($P<0{\cdot}001$ for the difference between the groups).

After 10–14 months, the mean fall in ESR since diagnosis was $42{\cdot}0 \pm 7{\cdot}85$ mm for the immunotherapy group and $14{\cdot}96 \pm 8{\cdot}5$ mm for the placebo group ($P<0{\cdot}001$ for the difference).

HIV SEROLOGY

Other than a pilot number who were tested in Kano by the ELAVIA test, the Wellcome HIV 1 test and the Division of Virology (UCL Medical School)

Table 3 Results of smear microscopy for acid-fast bacilli

	Immunotherapy						Placebo					
Sputum		0	+	++	+++	++++		0	+	++	+++	++++
At diagnosis	(90)	0	61·1	26·7	10	2·2%	(90)	0	52	35	12	1%
First follow-up	(75)	73·3	22·7	4	0	0%	(65)	18	62	18	2	0%
Second follow-up	(33)	66·6	24·2	6·1	3	0%	(26)	15	54	19	12	0%

The numbers in parentheses are patients' samples examined at each time (three samples taken on three successive days represents one sputum investigation). One *M. vaccae* recipient and two placebo recipients had missing data. 0, no acid fast bacilli seen on microscopy of 100 high power fields; +, scanty acid fast bacilli seen, but less than 1 per high power field; ++, 1–9 bacilli seen per high power field; +++, 10–99 bacilli seen per high power field; ++++, 100 or more bacilli seen per high power field.

test kit for HIV 2 were applied to the initial sera obtained from persons who were followed-up after 10–14 months, including those who had died. In the immunotherapy group, 5/34 (15%) patients were HIV 1 positive and none were HIV 2 positive. Of those that received placebo, 9/47 (19%) were HIV 1 positive and two of these were strongly positive for HIV 2 (an analysis of the results for those positive for HIV 1 is shown in comparison with those of the entire groups in Table 2). None of the five HIV 1 positive patients who received immunotherapy had died, only one of them still had AFB visible in the sputum at 10–14 months from diagnosis, and all were clinically well. In contrast, of the nine HIV 1 seropositive patients who received placebo, six had died, and the three survivors (including the two who were also HIV 2 seropositive) were all sputum positive for AFB and clinically suffering from advanced tuberculosis.

AGALACTOSYL IgG [%GAL(0)]

The difference between the results at diagnosis and at the first follow-up after intervention, corrected for the patients' ages, are shown in Table 1. Within 2 weeks of intervention, the immunotherapy recipients already showed a significant fall in %Gal(0) ($P<0{\cdot}0005$, paired t-test), whereas those in the placebo group show no significant change [$P<0{\cdot}05$ for the difference in %Gal(0) between the placebo and immunotherapy groups].

Discussion

Despite the many drawbacks experienced in the execution of this study under the adverse conditions prevailing in Kano, it is clear from the evidence provided that immunotherapy with *M. vaccae* was beneficial to the patients, and we believe resulted in a drop in mortality from pulmonary tuberculosis. Admittedly the physician in charge (PCO) was aware of the type of intervention given to the patients, but the patients themselves did not know which intervention they received. The laboratory tests were carried out in a blinded manner, as was the reading of X-rays, and these data provided objective support for the clinical findings recorded. The physician in charge did not participate in finding patients at follow-up, and the team finding the patients did not know which patient had received which intervention. The problems experienced with follow-up were not unexpected, though numbers traced 10–14 months after entry were fewer than we had hoped. The poor follow-up can be attributed to the lack of any real addresses, civic disruptions, and because many patients had returned to their homes in villages outside the city.

Hospital data compiled by the Ministry of Health in Kano indicate a high mortality rate amongst patients diagnosed as having tuberculosis. However, these records reflect deaths occurring during hospitalization. Deaths are unregistered in Kano, and cultural and religious rites facilitate quick burials, usually by relations of the deceased. Thus it is difficult to be sure of figures except for those occurring in hospital or amongst persons reliably traced in the 10–14 month follow-up. It could be speculated that the disproportion in the numbers followed-up in the two groups may be attributed to two factors. First, many of the deaths recorded in the placebo group may have occurred prior to the population disturbances, when larger numbers of both groups may still have been in the city. Secondly, rapid resolution of disease in the immunotherapy recipients may have resulted in their being fitter, and more able to leave the city than the placebo group.

Although the percentage of the patients followed-up at 10–14 months was low, the proportion of patients found at the first follow-up, approximately 20 days after starting chemotherapy, was satisfactory though slightly skewed by the four

patients in the placebo group who had died. At this time, several parameters indicating benefit from immunotherapy were recorded. There was more rapid recovery of body weight, disappearance of acid-fast bacilli from the sputum, accelerated fall of ESR, and a change in agalactosyl IgG. In contrast to the slight elevation of %Gal(0) seen in patients treated with chemotherapy alone, there was a fall in this glycosylation variant in the immunotherapy group. This slight elevation in %Gal(0) amongst patients who received chemotherapy alone has also been seen in patients receiving optimal chemotherapy in the UK (17), and shows that the changes occurring in the immunotherapy recipients are distinct from those that would be expected if they had received privileged chemotherapy. All patients had received the same type of chemotherapy, available from commercial drug stores. Agalactosyl IgG has been found to be elevated in tuberculosis, rheumatoid arthritis, Crohn's disease, and leprosy during episodes of erythema nodosum leprosum (ENL) (15). It is not elevated in acute rheumatic fever or in viral infections. This would suggest that it is not merely a correlate of inflammation or an acute phase response. It has been suggested that raised %Gal(0) occurs in conditions associated with chronic T-cell mediated response and with increased production of cytokines, especially IL-6 and TNF-*a*.

Chest X-ray examinations were performed by a radiologist working at the General Hospital in Kano City, who was not aware of which patient had received which intervention. He gave a qualitative assessment of the chest radiographs of patients seen at diagnosis and 1 month after diagnosis. In this respect, the extensive radiological evidence of tuberculosis seen at diagnosis (often with cavity formation), showed signs of early resolution after 1 month of chemotherapy in many of the patients randomly selected to receive immunotherapy. By the final follow-up, when X-ray examinations were performed on eight immunotherapy and 12 placebo recipients, improvement was much more obvious in the immunotherapy recipients, with the radiologist calling attention to remarkable closure of cavities in five patients and marked improvement in three patients in that group. At this time in the placebo group, radiology showed active progression of disease in eight patients with two showing no change and two others showing some resolution of apical cavities seen at onset.

The clinical features associated with the active intervention, though subjective, were indicative of the general trend of accelerated recovery, as shown by both fewer cases with lymphadenopathy

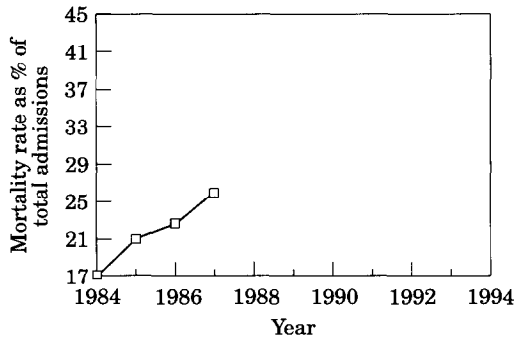

Fig. 1 Percentage *mortality* rates amongst tuberculosis patients in Kano State, Nigeria, 1984–1987. If the % mortality continued to rise at a similar rate, which is possible in view of the upsurge in HIV incidence and the worsening of nutritional level and social conditions, it would have reached more than 40% by 1992, when this study was performed.

($P<0{\cdot}004$) and splenomegaly ($P<0{\cdot}002$), and by the objective criteria of changes in weight and ESR, and disappearance of acid-fast bacilli from the sputum.

The patients' own assessments of their well-being is reflected in the duration of chemotherapy purchased. Although both groups were equally encouraged to buy treatment, survivors of the placebo group bought at least some drugs for, on average, twice as long as immunotherapy recipients did. Of course, we do not know how much chemotherapy was bought by those who died. Thus, it is even more significant that despite more intensive chemotherapy, 85% of the placebo group still had AFB visible in their sputum 10–14 months after entry into the trial.

The extremely poor results obtained with chemotherapy alone (placebo group) may be attributed to two factors (18). First, these patients may have taken intermittent doses of the cheaper drugs (streptomycin and isoniazid), rather than what was prescribed. Secondly, it is well documented that 50–70% of the drugs available for purchase in Kano are adulterated, out of date, or fake (3,13,14). It is striking that these deficiencies were made good by immunotherapy. The poor results obtained in the placebo group are not improbable for this part of Africa. Until 1987, the Kano State Infectious Diseases Hospital (IDH) kept statistics for deaths from tuberculosis and when the last few years of these are projected to 1992 (assuming the mortality rate continued to increase at the same rate), the expected mortality would be 40% (Fig. 1). Moreover, this projected figure is not corrected for excess mortality attributed to the rapid increase in HIV over the same period.

Despite the apparent advantages of immunotherapy, 33% smear-positivity 1 yr after diagnosis is hardly acceptable and unfortunately, there are no culture facilities available for mycobacteria in Kano. A study recently carried out in Lagos (19) on patients who remained sputum positive after at least 6 months of chemotherapy, reported a 56% prevalence of resistance to one or more drugs (isoniazid 38%, streptomycin 13%, rifampicin 2% and ethambutol 3%). Other studies with *M. vaccae* suggest that more than one dose may be necessary for the treatment of patients infected with drug-resistant bacilli (20). Thus, it is possible that those patients who remained smear positive (33%) were infected with drug-resistant strains. In support of this conclusion, the 11 patients making up the 33% were sputum positive at all three assessments, suggesting that they may have been harbouring resistant bacilli from the beginning.

The data suggest that beneficial effects of immunotherapy occur within the first 2 weeks or so of its injection, and that patients with persisting sputum positivity beyond this time might benefit from a second dose. We hope to explore this possibility in studies currently starting.

The number of patients co-infected with HIV 1 or HIV 2 was more than expected, particularly in a country with historically low seroprevalance rates for HIV (21), in comparison with the rest of the African continent. Even with these small numbers, survival was significantly better in the immunotherapy recipients, with patients doing just as well as the HIV negative patients (22).

In earlier studies, 1 month (9,10) or 6 weeks of chemotherapy was given before immunotherapy with *M. vaccae*. This was to enable the numbers of live bacteria to be substantially reduced before attempting an immunological manipulation. Our data showing that the injection is just as effective when given after 1, 2 or 3 weeks of chemotherapy, suggests that the wait may not be necessary. Since survival seems to be a parameter improved by immunotherapy, there are obvious advantages in giving it as soon as possible.

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